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Substrate influence and temporal changes on periphytic biomass accrual and metabolism in a tropical humic lagoon

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Abstract

We performed a field experiment in a tropical humic coastal lagoon to evaluate periphyton biomass accrual and metabolism on three different substrates (1) plastic ribbons, (2) green and (3) senescent leaves of the emergent macrophyte *Typha domingensis*) over 30 days. The contribution of autotrophic biomass decreased as total biomass increased over the time. Mean periphytic ash free dry weight ranged from 0.8 to 5.6 mg cm^{-2}, but periphyton chlorophyll *a* concentrations presented shorter amplitudes, which oscillated from 0.12 to 0.44 μg cm^{-2} throughout the experiment. Periphyton metabolism was overall heterotrophic on all substrates, especially on senescent leaves. Our data show that substrate type influenced both biomass accrual and periphyton net productivity and respiration rates throughout periphyton development and highlighted the dominance of heterotrophic metabolism. The periphyton respiration may be subsidized by both water- and substrate-derived allochthonous energy pathways, shedding light on the role of periphytic assemblages to the carbon cycling, as a source of CO_2 to the system.

Keywords: Periphyton; Heterotrophy; Humic lakes; *Typha domingensis*; Carbon cycle

Introduction

The periphyton assemblage is as an important component of ecosystem functioning, as it can scavenge large amounts of N and P from the water column (Blumenshine et al. 1997), provide an easily available nutritious food source for grazing invertebrates (Fink and Von Elert 2006) and perform as a key component of lake metabolism, being an important primary producer and hence an important CO_2 sink of aquatic ecosystems (e.g. Wetzel 1963, 1990; Goldman 1981; Burkholder and Wetzel 1989). However, despite its undoubted importance in aquatic systems, its relationship with the substrate is very controversial (Wetzel 1983). A huge debate has arisen about the substrate influence on many aspects of periphytic assemblage – especially on biomass and taxonomic composition. Morin (1986) found that the natural substrate can have positive effects on the total number of algae, and Putz (1997) found that the

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community that grows on natural substrate was less limited by phosphorus. However, Cattaneo and Kalff (1979) found no difference in terms of biomass when comparing periphyton on artificial and natural substrates, and Fairchild and Everret (1988) observed that natural substrates had no quantitative or qualitative effect on periphytic algal composition. In this context, few studies have evaluated the substrate influence on periphytic metabolic features (but see Vadeboncoeur et al. 2006). Understanding the nature of autotrophic and heterotrophic processes is central to the study of biogeochemical cycles (Biddanda et al. 2001), and determining the balance of this processes in periphytic communities may be crucial for our understanding of whole ecosystem metabolism and functioning.

In coastal lagoons, the high perimeter:volume ratio and the shallowness provides favorable conditions for the development of a large littoral region (Kjerfve 1994). Macrophyte species, which segregate along the littoral-zone slope, continuously provide new substrata for periphyton colonization (Wetzel 1990; Schindler and Scheuerell 2002). Such diversity of substrata was pointed out to be a source of natural variability on periphytic biomass (Morin and Cattaneo 1992; Burkholder 1996). Coastal lagoons also offer favorable conditions for heterotrophy, due to their typically high concentration of humic dissolved substances and macrophyte shading, which result in both high availability of organic carbon and attenuation of the sub aquatic active photosynthetic radiation (Grimshaw et al. 1997; Jonsson et al. 2003). Few studies have explicitly evaluated the metabolism of periphytic communities in lakes where environmental conditions should favor heterotrophy, such as in cases of oligotrophy and low light availability. The relative biomass, as well as the magnitude of carbon flux through bacteria, are expected to be larger in oligotrophic than in eutrophic water bodies (Biddanda et al. 1994; Cotner and Biddanda 2002), thus favoring the heterotrophic primacy. In addition, light is the only energy source for all photoautotrophic organisms, and therefore, light limitation may be as important as nutrient limitation for autotrophs (Hill 1996).

We conducted a manipulative field experiment to examine the biomass accrual and the metabolism of the periphytic community on natural and artificial substrates in a humic coastal lake. The main objective of this study was to assess substrate influences and temporal changes in periphytic biomass, net productivity and respiration rates in a tropical coastal lagoon.

Material and methods

Study area

This study was carried out in Cabiúnas Lagoon (22°15′S, 41°40′W), located at Restinga de Jurubatiba National Park, Rio de Janeiro – Brazil. Cabiúnas is a pristine coastal lagoon surrounded by a natural 'restinga' ecosystem which is characterized by coarse-sand soil and bushy vegetation. Its highly permeable watershed and dentritic morphometry favor a great input of terrestrial dissolved organic matter into the lagoon (Farjalla et al. 2002). According to Panosso et al. (1998) it has a surface area of 0.34 km^2, a mean depth of 2.37 and 4.48 of perimeter development index (Dp). According to Wetzel (1983) Dp values above 3.0 are considered elevated, and thus representing a high perimeter:surface ratio. It is a freshwater waterbody and the water is classified as humic and slightly acidic (pH 6.3) with an average temperature of 23.6 °C and mean concentrations of dissolved phosphorus less than 1.0 μM. According to Esteves et al. (1983), Cabiúnas Lagoon may be classified as a dark water lagoon, due to high concentrations of humic compounds which are a result of the decomposition of organic matter produced in great amounts in it drainage area and by the marginal vegetation. The littoral zone supports dense stands of an emergent macrophyte *Typha domingensis* (Pers.).

Experimental set-up and chemical analysis

Plastic ribbons and the emerged part of green and senescent leaves of *T. domingensis*, randomly selected, were chosen as substrates for periphyton growth. Senescent leaves were chosen because senescent shoots or leaves of most emergent aquatic macrophytes start to decay without detachment, resulting in the presence of large crops of dead standing plant detritus during much of the year (Newell 1993), and therefore, a highly available substrate for periphyton colonization in the Cabiúnas lagoon. Emergent parts of macrophyte leaves were cut (detached) at the water surface level, to avoid previous periphyton colonization.

Detached leaves and plastic ribbons of 20-cm length were attached to wooden structures to keep them in a horizontal position, parallel to the water surface. Previous analyses showed that such wooden structures do not leach organic matter into the water which could influence the outcome of this study (data not showed). Although plant leaves are naturally positioned in a vertical orientation, we chose the horizontal position to keep light evenly distributed along the length of the leaves, thereby decreasing variability due to depth-light attenuation. The wooden structures were buried in the sediment and the substrates were kept at a depth of ca. 0.3 m in an open area close to a stand of *T. domingensis*. Secchi disk depth was 1.1 m in this area and water column depth was ca. 2.5 m. This study was carried out for 30 days (February 6–March 8, 2003).

The experiment started with nine replicates for each substrate, and the sampling was carried out by removing three replicates from each substrate every sampling day.

The colonized substrates were collected after 4, 15 and 30 days of incubation. The leaves and the ribbons were carefully sampled and individually transferred to the lab into underwater chambers filled with lagoon water to minimize periphyton damage.

Water temperature, electrical conductivity (thermo-conductivimeter multi-functional probe YSI-30), depth, pH (pHmeter Analion-2000) and dissolved oxygen (portable oxymeter YSI-95) were measured every sampling day at the study site. Water samples were also collected for analysis of dissolved organic carbon (Total Carbon Analyzer TOC 5000 Shimadzu), and total nitrogen and phosphorus concentrations. Total nitrogen was measured after persulfate oxidation and nitrate reduction in a cadmium column with post nitrite determination in a flow injection analyzer (APHA 1989). Total phosphorus was measured with the ammonium-molybdate method after persulfate oxidation (Mackereth et al. 1978).

In the laboratory, the substrates were carefully transferred into a 5 l container with lagoon water. The substrates were kept under constant aeration and artificial light incidence of 200 μW cm^{-2} for 2 h for the purpose of periphyton stabilization. This light intensity was similar to the sunlight intensity at the incubation depth at noon, and it has been found to be the optimal light intensity for periphyton productivity, observed in previous experiments in the same lagoon (Guariento et al. in preparation).

We measured periphytic net community productivity (NP) and respiration rates (RR) with a Clark type dissolved oxygen microsensor, originally described by Revsbech et al. (1981). The 90% response time of the sensors was 0.2–0.6 s, and the sensitivity to stirring was below 3%. The sensors were positioned by a manual-driven micromanipulator and the current sensor was measured with a picoammeer connected to a strip chart recorder. Net community productivity was obtained through O_2 diffusion fluxes from the substrates upper face obtained from O_2 profiles in light conditions, while respiration was obtained in dark conditions. O_2 concentration within the biofilm was measured in 50 μm depth increment until the measurement approaches to zero. The flux of O_2 out of the photic zone is the sum of the flux toward the overlaying water and the flux toward the deeper heterotrophic layers of the biofilm. Steady state O_2 profiles were confirmed by comparing two or more profiles measured within a time interval of 10–30 min. The diffusion flux [$J(x)$] of O_2 was calculated by Eq. (1), Fick's first law of diffusion:

$$J(x) = -\Phi D\mathrm{s}(x)\frac{\delta C(x)}{\delta(x)}, \quad (1)$$

where $C(x)$ is the concentration of O_2 at depth x, Φ the porosity, and D_s the apparent diffusion coefficient of oxygen. ΦD_s was assumed to be 1.44×10^{-5} cm^2 s^{-1}.

Four profiles, 5-cm apart from each other along the substrate main axis were performed in each substrate unit and the measurements for each replicate were averaged for post statistical analysis. This procedure eliminated the tendency to select areas with an obvious periphyton mat and then overestimate the productivity (Bott et al. 1997). Periphytic gross primary productivity (GPP) was estimated by summing NP and RR. This procedure does underestimate GPP because RR is measured in the dark and photorespiration is not considered, however it is a very reasonable procedure to estimate the GPP.

After performing, NP and RR measurements, the periphyton from the upper face of leaves and ribbons were carefully scraped with a razor blade and filtered through a GF/F Whatman filter (0.7 μm pore size) for chlorophyll *a* (CHL*a*) and ash free dry weight (AFDW) determination. We did not observe any detrital component of *T. domingensis* in the samples after periphyton removal. So, it is unlikely that any macrophyte detrital components could be included in the periphyton bulk biomass. Chlorophyll *a* was determined with a spectrophotometer after extraction with boiling ethanol. AFDW was determined after drying the GF/F filter at 70 °C for dry weight determination and later ashed at 500 °C during 1 h for ash determination. Periphyton samples were scraped in triplicates to determine total phosphorus (P) and total nitrogen (N) content. The slurry of scraped periphyton was adjusted to a defined volume with deionized water, and then the nutrient content analyses were performed in the same way as the water analysis described above. Leaf and ribbon scraped areas were also determined.

Data analysis

Differences in the measured parameters in relation to exposure time and type of substrates were compared using bi-factorial ANOVA followed by a Tukey HSD test. Independent factors comprised substrates ($N = 3$) and time ($N = 3$). Log-transformed variables were used throughout to reduce the observed heterogeneity in the variance.

Correlation analyses were used to explore interdependence among variables. A Pearson correlation was applied to examine the existence of the following relationships: (1) periphyton community NP vs. RR, to assess the relative balance between production and consumption of organic matter within periphytic communities; and (2) periphyton biomass vs. CHL*a*:-biomass ratio, to assess the contribution of autotrophs within periphytic communities. All statistical analyses were performed using the statistical program STATISTICA 6.0.

Results

The limnological parameters in the sampling site during the study period are presented in Table 1. Total P concentration was less than 1 μmol L^{-1} at the experimental site but high concentrations of dissolved organic carbon were observed, above 20 mg L^{-1} on average.

There was a significant interaction between time and substrates when determining periphyton AFDW responses in the present study (ANOVA, $P<0.05$). Senescent leaves showed a significant increase in periphyton AFDW from day 4 to day 15 (threefold) and from day 15 to day 30 (Tukey, $P<0.05$), while green leaves and artificial substrates showed a significant increase in periphytic AFDW only after 30 days of incubation (Tukey, $P<0.05$, Fig. 1A). The CHL*a* content presented a significant increase over time, but it was not related to the substrate type (ANOVA, $P<0.05$). From day 4 to day 15, the CHL*a* content of the periphyton in the plastic ribbon and green leaves ranged from 0.16 to 0.26 and 0.10 to 0.23 μg m^{-2}, respectively, although no significant difference was observed from day 15 to day 30 (Tukey, $P<0.05$). The CHL*a* content in senescent leaves was stable from day 4 until the end of the experiment (Fig. 1B).

Periphyton RR where significant different regarding substrate type and varied throughout the experiment (ANOVA, $P<0.001$), with the lowest and highest values observed in artificial and senescent leaves, respectively (not detectable and 0.72 nmol O_2 cm^{-2} s^{-1}, respectively, Fig. 2A). The NP was generally negative over the exposure time, which indicated the prevalence of heterotrophic metabolism. However, a positive net balance of O_2 flux in the biofilms on green leaves and on artificial substrate was observed on day 15 (Fig. 2B), evidencing the significant interaction between substrates and time on NP response (ANOVA, $P<0.001$). The NP on day 30 was significantly lower in senescent leaves (-0.46 nmol O_2 cm^{-2} s^{-1}) (Tukey, $P<0.05$). The GPP showed a trend of increase throughout the period of periphyton biomass accrual. On days 15 and 30, GPP was significantly higher in green and senescent leaves than in artificial substrate (Tukey, $P<0.05$), but no differences were observed between the natural substrates (Tukey, $P>0.05$, Fig. 2C).

Analyzing biofilm oxygen profiles, we observed that oxygen distribution along biofilm depth was consistently generated by an internal biofilm process. An advantage of the method used in this experiment to assess periphyton metabolism (oxygen profiles using highly sensitive clark-type sensors) is the fine-scale spatial (50 μm) and temporal resolution of the oxygen measurements. Measuring the oxygen concentration along the biofilm depth (periphyton) we are able to identify oxygen production zones. On the senescent substrate where NP was negative, oxygen concentration decreased

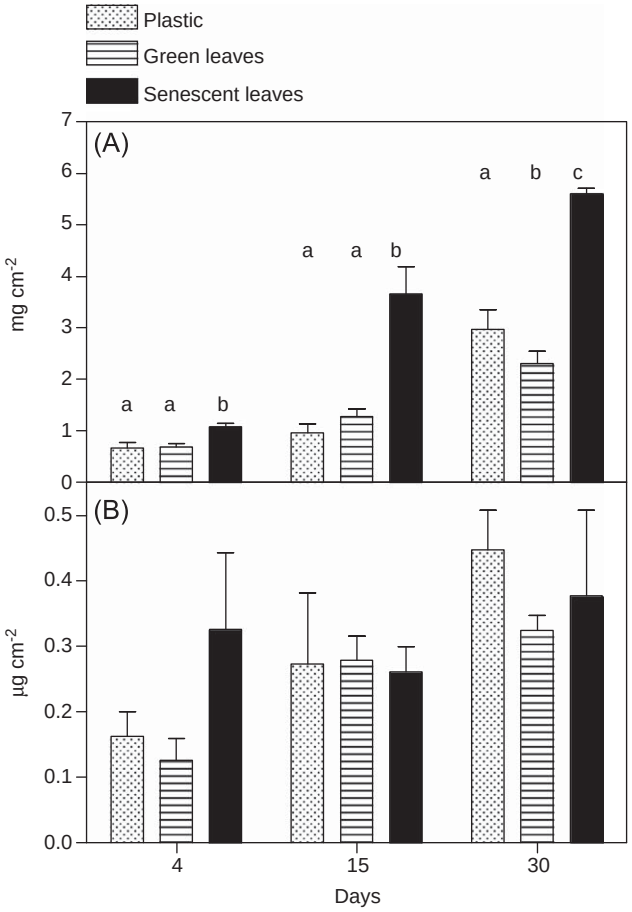

Fig. 1. Ash free dry weight (A) and chlorophyll *a* (B) on plastic substrate, green and senescent leaves of *T. domingensis* throughout the study period. Letters above graph bars (a, b or c) indicate statistical differences among substrate types within each sampling day, equal letters indicate no difference and different letters indicate significant differences (Tukey, $P<0.05$). Each bar represents the mean + 1 S.D. (No significant difference was observed for chlorophyll *a* measurements within each sampling day.)

Table 1. Mean values and standard deviations of water parameters in the sampling site during 30 days of experiment

	Mean (S.D.)
Water temperature (°C)	28.5 (0.5)
Transparency as Secchi disc depth (m)	1.1 (0.05)
Depth (m)	2.2 (0.01)
Total P (μmol L^{-1})	0.86 (0.03)
Total N (μmol L^{-1})	72.53 (6.46)
Dissolved organic carbon (mg L^{-1})	23.7 (0.2)
Conductivity at 25 °C (μS cm^{-1})	560.5 (2.5)
pH	6.3 (0.6)
Dissolved oxygen (mg L^{-1})	5.4 (1.2)

Samplings were done at the beginning of the experiment and every 10 days until its end ($n = 4$).

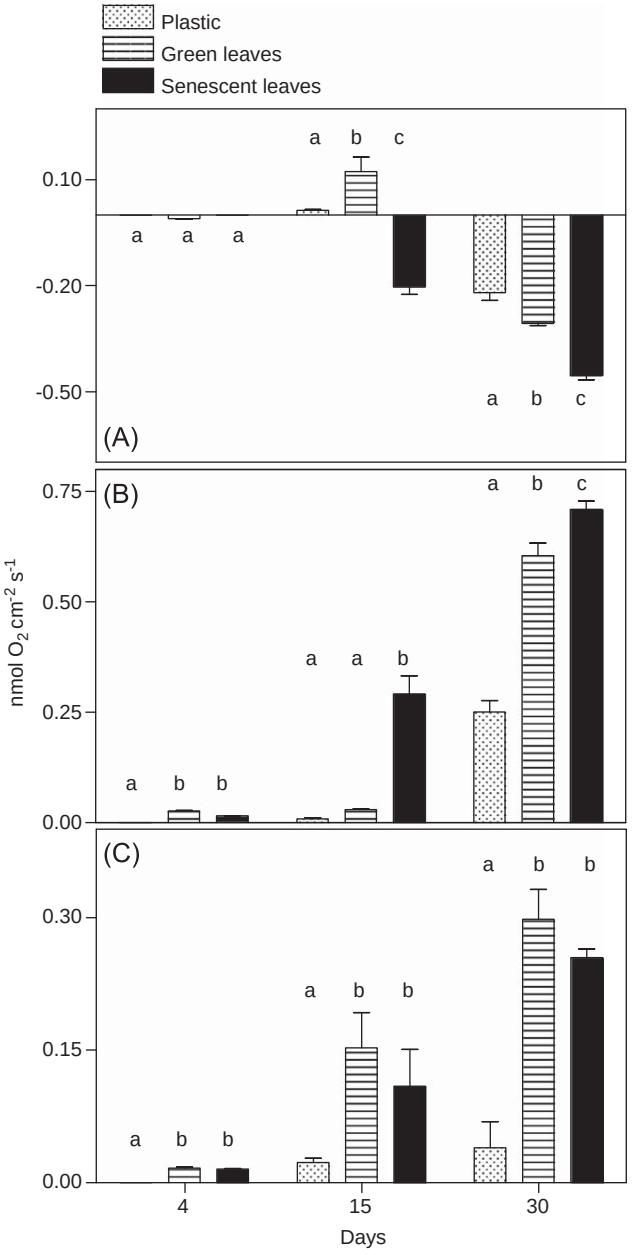

Fig. 2. Net community productivity (A), respiration rates (B) and gross primary productivity (C) of periphyton on plastic substrate, green and senescent leaves of *T. domingensis* throughout the study period. Letters above graph bars (a, b or c) indicate statistical differences among substrate types within each sampling day, equal letters indicate no difference and different letters indicate significant differences (Tukey, $P<0.05$). Each bar represents the mean + 1 S.D.

monotonically with biofilm depth (Fig. 3). Our results clearly indicate that for those substrates where NP is positive, there is a hump-shaped pattern of oxygen concentration along the biofilm depth (Fig. 3). The increase in the oxygen concentration along biofilm depth is due to the oxygen production by autotrophic organisms within the biofilm and their subsequent decrease is caused by oxygen heterotrophic consumption. The substrate could not contribute to the slope pattern, because of the monotonic decrease in the oxygen concentration from the maximum concentration at 0.5 mm depth until the substrate surface. Oxygen production by the macrophyte leaf (at the bottom of the biofilm) would change the profile and another increasing slope of oxygen concentration would be observed (or at least no oxygen concentration decay), generating a bimodal curve of oxygen concentration (two production zones) along the biofilm depth. There is other evidence that corroborates with the fact that macrophyte leaves are not contributing to our periphytic metabolism measurements. Comparing artificial and green substrate profiles, we observed that the oxygen maximum concentration is practically at that same biofilm depth. Since artificial substrates do not produce oxygen, it is reasonable to believe that both profiles (artificial and green leaf) are generated by the same processes (within biofilm autotrophic production). These results validate our experimental setup and assure that macrophyte leaves could not be contributing to the periphytic metabolism measured in our experimental system.

There was a significant negative correlation between periphyton NP and RR (Pearson $r=-0.92$, $P<0.001$, Fig. 4), suggesting that changes in the net metabolism was mainly attributed to changes of heterotrophic activity. The CHLa:biomass ratio and biomass accrual also showed a significant negative correlation (Pearson $r=-0.75$, $P<0.001$, Fig. 5). This result is evidence of the decreasing contribution of autotrophs as periphytic biomass increases.

Discussion

Periphyton algal biomass increased with exposure time in all treatments, although it showed short-range variation throughout the experiment. This may be attributed to the low nutrient availability in the water column, especially phosphorus (Table 1). Phosphorus is a key component regulating autotrophic biomass and growth in freshwater ecosystems (Hansson 1992), as was pointed out to be the prime factor affecting wetland periphyton structure and function (McCormick et al. 2001). Previous studies in Cabiúnas lagoon showed that phosphorus is the major limiting nutrient regulating planktonic bacteria (Farjalla et al. 2002) and therefore, the low phosphorus availability may be regulating periphyton algae as well. Light is the only energy source for all photoautotrophic organisms and therefore, light limitation may be as important as nutrient limitation for primary producers (Hill 1996). Hill and Knight (1988) reported that in shaded conditions, benthic algal biomass did not increase significantly, indicating the

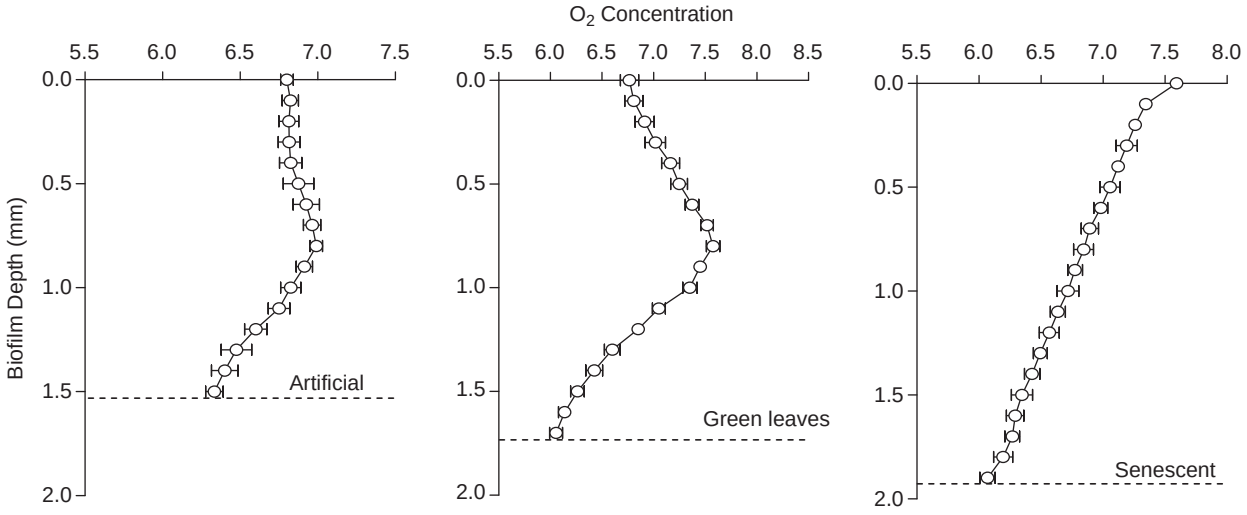

Fig. 3. Oxygen profiles at day 15. Each graph corresponds to one substrate type. Each point corresponds to the mean ± S.D., $N = 3$. The dashed line represents the substrate surface.

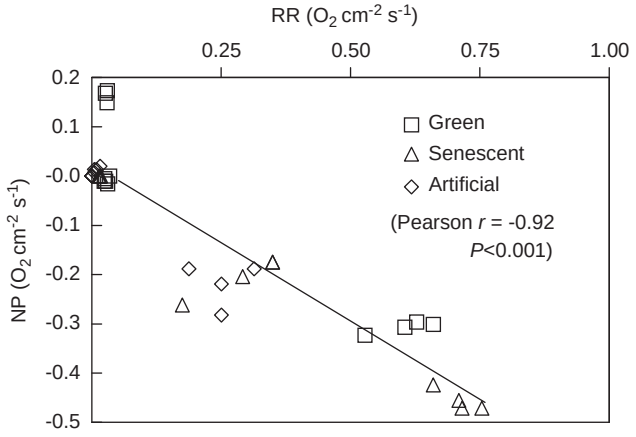

Fig. 4. The relationship between net community productivity (NP) and respiration rates (RR) of periphytic communities ($n = 36$) colonizing three different substrates throughout the study period.

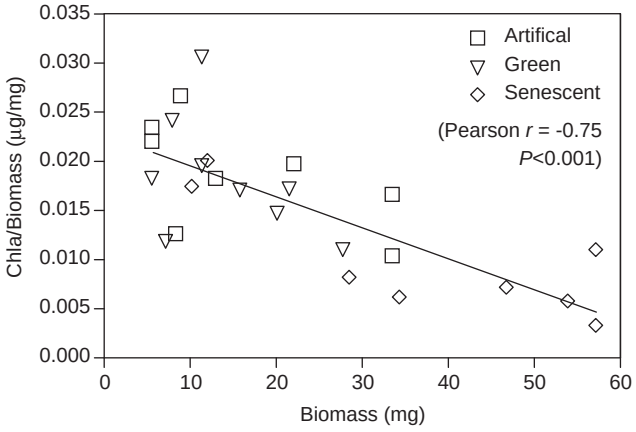

Fig. 5. The relation between chlorophyll *a*:biomass ratio and biomass of periphytic communities ($n = 27$) colonizing three different substrates throughout the study period.

overriding importance of shading as a factor limiting algal growth in the face of nutrient limitation. Thus, light limitation can be particularly strong for periphytic autotrophic biomass, due to absorption of light by the water column, and hence, influence not just autotrophic biomass but also the balance between autotroph to heterotroph ratios (Mosisch et al. 1999). Herbivory may also play an important role regulating periphytic algal biomass, although not measured in this experiment. However, experimental studies conducted with benthic algal communities showed that grazer limitation is weak compared with nutrient limitation at low productivity sites (Darcy-Hall 2006). Thus, grazer regulation of benthic algal biomass is expected to be weak in this experiment.

The AFDW increased on all substrates in response to exposure time, asserting that periphyton biomass is a function of the biofilm age (Christenson and Characklis 1990). In this study, no significant difference in periphytic biomass was found between green leaves and the artificial substrate, although significant differences were found between these substrates and senescent leaves. When natural substrates are aging, death fragments could detach from the macrophyte leaf and be included in periphyton bulk biomass, especially for senescent leaves. However, we refrain to believe that this artifact could account for our results. Detachment of death fragments is expected to be variable among replicates, generating high deviations from the mean periphyton biomass, a phenomenon not observed in this

experiment (Fig. 1). In addition, using a scope, we did not observed any detrital component of *Typha* leaves in the samples after periphyton removal from the substrate.

The importance of substrate texture was documented as an important feature shaping periphyton communities (Dudley and D'Antonio 1991), especially differences in the texture of macrophyte leaves (Morin 1986). Senescent leaves of *Typha* have a quite wrinkled surface that may facilitate colonization by auto- and/or heterotrophic organisms. In addition, Mann and Wetzel (1996) showed that senescent culms of *Typha latifolia* released more dissolved organic carbon than green culms during the decomposition process. Hence, differences in surface texture and the release of organic compounds could explain the highest periphytic biomass in senescent leaves. However, this is only a partial framework to explain the plant–host interactions. In a recent study, Gallardo-Willians et al. (2002) isolated several compounds from aqueous extracts and leachates of *T. domingensis*, among them, various phenolic acids. Many allelopathic interactions in aquatic systems occur as surface-associations, either in benthic communities or between photoautotrophs and their epiphytes (Gross 2003), preventing or decreasing periphyton colonization. Although allelopathic effects on epiphytic density and productivity cannot be excluded, we cannot assure allelopathy as a mechanistic factor determining differences between the substrates used in this experiment. The release of allelopathic compounds were observed for both green (Gallardo-Willians et al. 2002) and senescent (Maie et al. 2006) *Typha* leaves and the periphytic community on the plastic substrate never achieved higher biomass than those found on natural substrates. Further studies are necessary to evaluate the quantitative and qualitative differences in the release of allelopathic substances of green and senescent *Typha* leaves and their consequences on periphyton colonization.

Duarte and Cebrián (1996) pointed out that most coastal ecosystems dominated by macrophytes are highly productive and overall autotrophic. Littoral zone production has often been found to dominate total lake production, especially in shallow lakes where a large part of the lake's surface area lies within the littoral zone (Loeb et al. 1983). Wetzel and Søndergaard (1998) showed that periphyton production in such shallow systems may exceed that of aquatic macrophytes. However, in contrast to the extensive literature about the importance of periphytic communities as carbon dioxide sinks in aquatic ecosystem (Wetzel 1963; Goldman 1981; Burkholder and Wetzel 1989), our data sheds light on the unappreciated role of periphyton as a carbon dioxide source. Despite the increase in GPP, the NP decreases over time, presenting negative values in the end of the experiment. The significant negative relationship between NP and RR (Fig. 4) indicates that the decreasing NP is attributed to higher microbial respiration, which outperforms the oxygen produced by photosynthesis. Respiration measurements and other studies comparing carbon flow have shown that prokaryotic heterotrophic production can exceed total autotrophic production at any given point in time (Ducklow and Carlson 1992). So, bacterial production is not necessarily constrained to be less than primary production (del Giorgio et al. 1997). The periphyton accrual biomass was followed by the diminishing contribution of autotrophs within the biofilm (Fig. 5). The increase in heterotrophic dominance may be due to the better ability of bacteria to acquire nutrients than algae in oligotrophic conditions, conferring the highest biomass on bacteria (Sanders et al. 1992; Cotner and Biddanda 2002). In addition, it is presumable that, in humic lakes, periphyton may experience light limitation even in shallow depths, thereby favoring its heterotrophic metabolism. In fact, even when the demand for carbon was higher than its production (i.e. negative NP) the periphyton biomass increased over time. These data suggest that allochthonous sources of energy may be subsidizing periphytic heterotrophic activity, resulting in an increase in biomass that could not be supported by autochthonous pathways alone.

Lakes in general have been implicated as potential sources of CO_2 into the atmosphere (Cole et al. 1994), and an important factor driving the balance between auto- and heterotrophy is the availability of dissolved organic carbon – DOC (Hanson et al. 2003). Autotrophy prevails in lakes where DOC concentrations are low, whereas high DOC concentration leads to heterotrophy (Praire et al. 2002). Such DOC may originate from allochthonous sources and can support heterotrophic metabolism in rivers (Cole and Caraco 2001) and in lakes (Pace et al. 2004).

In the present case, two external sources of energy may be subsidizing periphyton heterotrophy:

(1) *Carbon availability in the surrounding water column*. The high carbon concentration in the water column, as DOC, can be exploited by periphytic bacteria and reverberate on bacterial biomass and periphyton metabolism. However, allochtonous humic substances are usually characterized by high C/N molar ratios related to intense diagenetic processes during transport from the surrounding landscape to aquatic ecosystems (Aitkenhead-Peterson et al. 2003). DOC bioavailability is directly related to the diagenetic state of carbon compounds, and such molecules previously degraded by microbial activity would be less susceptible to further bacterial attack (Amon and Benner 1996). In the face of evidence showing that external sources of DOC may have modest effects on bacterial metabolism, the increase in the allochthonous DOC contribution to lake total

carbon budget was pinpointed as an important driving factor in subsidizing lake metabolism, leading to heterotrophy (Pace et al. 2004; Biddanda and Cotner 2002). In addition, a recent whole lake experiment showed that terrestrial DOC provides substantial support for microbial respiration (Cole et al. 2006). Tropical coastal lagoons are subjected to high inputs of colored DOC during the rainy season (Farjalla et al. 2002), which enhances the lake bulk DOC and attenuates the subaquatic active photosynthetic radiation. Therefore, this carbon amendment may affect periphyton metabolism as well, possibly intensifying its heterotrophy.

(2) *Carbon release from the substrates*. It was previous observed that there is a release of carbon molecules throughout the decomposition processes of *Typha* detritus (Mann and Wetzel 1996), especially in the case of senescent culms. This additional DOC may support periphyton biomass. Thus, the highest RR observed in the natural substrates (particularly on senescent ones), and consequently lower NP, may also be attributed to an additional carbon input supported by substrate decomposition.

The present investigation showed that biomass and metabolic features of periphyton assemblages are influenced by substrate identity. We suggested that periphyton were influenced by the release of labile compounds by the substrates, substrate surface texture and resource availability of ambient water. Our data also indicate that periphyton may act as a CO_2 source to the system and may be supported by multiple allochthonous energy pathways. These findings are particularly important for the functioning of wetlands and shallow lakes, where the periphytic biomass is generally high and the littoral–pelagic and land–water connectivity assume greater importance for subsidizing ecosystem-level process such as aquatic metabolism (Scheffer 1998; Schindler and Scheuerell 2002). In these systems, further investigations should focus on evaluating the actual contribution of periphyton communities to lake metabolism as well as the response of periphyton metabolism to natural seasonal DOC inputs into the ecosystem.

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